WP 1.1: Mode of delivery and gut microbiota in term infants

1. Related WPs, MG contact person: Synergies with WP 1.2b,1.3a,1.3b,1.6,2.2,3.1. MG contact: Dennis Nielsen

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):
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3. Main aim / sub-aims:
a) To document if delivery method influences infant gut microbiota colonization and composition, short term and more long term
b) If a) is verified, to test if cesarean delivery results in a less diverse gut microbiota with less gene richness compared to vaginal delivery
c) If a) is verified, to test if gut microbiota colonization, composition and gene richness is related to later obesity development, metabolic syndrome or impaired immunity.

4. Background and a central hypothesis:
It is well-known that caesarean delivery is correlated to increased risk of impaired immunity, obesity and metabolic syndrome. Whether the increased risks are due to pre-operative antibiotic use before caesarean section (WP1.2b), due to other maternal risk factors predisposing to caesarean section, to postnatal nutrition (e.g. extent of breast-feeding) or to the caesarean section per se is not explored in detail. Mode of birth (caesarean, vaginal) is therefore hypothesized to exert long term influences on the infant gut microbiota, leading to impaired immunity and to produce excess growth. This hypothesis is supported by a recent study using only a small sample size (Jakobsson et al.; 2013, n = 9 and 15 for caesarean section and vaginal delivery, respectively) and 454/FLX-based 16S rRNA gene amplicon sequencing. This study found that infants born by caesarean section have lower gut microbiota diversity and moderately lower levels of some Th1-associated chemokines (CXCL10, CXCL11). Moreover, the vulnerability in outcomes after caesarian delivery may be related to specific genotypes.
We hypothesize that delivery-associated gut colonization has long term effects on colonization and development of immunological and metabolic disorders. Subgroups of individuals, with specific genotypes, are more susceptible for altered gut microbiota patterns leading to impaired phenotypes.

5. Key analyses and methods:
Fecal samples from infants and mothers at 0, 4 and at 12 months after birth are frozen at -80 ^{o}C (Halmstad Hospital, n=150 caesarean, n=320 vaginal). Food patterns are recorded. Metagenomic analyses (complete genome sequencing and SNPs for polymorphisms) will show how the infant gut microbiota colonization and development is influenced by delivery method, maternal gut microbiota, food patterns and antibiotics during the first year of life and how this relates to specific human genotypes.

6. Expected results:
All vaginal delivered pairs are analyzed but no caesarean pairs are yet investigated. Within the coming year we will have an answer to the question if caesarean delivered children have a less diverse gut microbiota composition and lower gene richness and how this relates to weight development and immunity. In addition to microbial colonization of the gut, other factors such as less breast-feeding in mother-child pairs where caesarian delivery is performed or more overweight mothers asking for caesarean delivery possibly play a role here. Human DNA for candidate genes will be analyzed the coming 1.5 year and correlations to gut microbiota colonization, composition and gene richness will be carried out.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol	x																							
Sample collection	x	x																						
Metagenomics	x	x	x		x	x	x	x	x	x														
Metabolism/growth			x	x	x	x	x	x	x	x														
Immunity parameters					x	x	x	x	x	x														
Genotype (in planning)							x	x	x	x	x	x												
Publication(s)										x	x	x	x	x	x	x								

8. Estimated budget from NEOMUNE: 1.3 mio DKK

The amount covers metagenomics analyses in collaboration with BGI-Shenzhen for this and potential other NEOMUNE projects. Significant co-funding from BGI is required (to be negotiated) and further funding is being sought.

9. Estimated budget from elsewhere: 2.5-3.5 mio DKK

2013 ALF J Dahlgren 300,000 SEK, Region Halland 300,000 SEK, Svenska Läkarsällskapet 100,000 SEK
2014 ALF J Dahlgren 300,000 SEK, Region Halland 300,000 SEK, Frimurare 100,000 SEK
2015 Vetenskapsrådet J Dahlgren 700,000 SEK
2016 Vetenskapsrådet J Dahlgren 700,000 SEK
Co-funding/other sources 0.9-1.9 mio DKK

10. Additional comments:

- All sample collection is carried out by 2013 and the main limitation is sample analysis capacity. The study is partly sponsored from other sources, but NEOMUNE provides supporting funds for microbiome analyses at BGI-Shenzhen and DNA analyses in Copenhagen.
- End-point data (weight development, immunity) etc. will be collected until 3 or 5 years of age
- The project is relevant for the NEOMUNE study parts (infants, pigs, mice) that investigate birth methods/exposure to microorganisms at and after birth, exposure to antibiotics around birth and study effects on gut colonization and immunity.
- The project provides bridging between the -omics analytical capacity at BGI also with other projects in NEOMUNE, particularly the studies being performed in China (WPs 1.3, 1.6). BGI is involved also in WP 2.3 in piglets on the epigenetic characterization of the gut responses to the first feed and microbiota (0.7 mio DKK funds allocated).
- Method(s) for human genotyping not yet fully decided on. Might involve SNP chipping, exome sequencing and low pass whole genome sequencing.